


Editorial

# H<sub>2</sub>S, Polysulfides, and Enzymes: Physiological and Pathological Aspects

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**Abstract:** We have been studying the general aspects of the functions of H<sub>2</sub>S and polysulfides, and the enzymes involved in their biosynthesis, for more than 20 years. Our aim has been to elucidate novel physiological and pathological functions of H<sub>2</sub>S and polysulfides, and unravel the regulation of the enzymes involved in their biosynthesis, including cystathionine β-synthase (EC 4.2.1.22), cystathionine γ-lyase (EC 4.4.1.1), thiosulfate sulfurtransferase (rhodanese, EC 2.8.1.1), and 3-mercaptopyruvate sulfurtransferase (EC 2.8.1.2). Physiological and pathological functions, alternative biosynthetic processes, and additional functions of H<sub>2</sub>S and polysulfides have been reported. Further, the structure and reaction mechanisms of related enzymes have also been reported. We expect this issue to advance scientific knowledge regarding the detailed functions of H<sub>2</sub>S and polysulfides as well as the general properties and regulation of the enzymes involved in their metabolism. We would like to cover four topics: the physiological and pathological functions of H<sub>2</sub>S and polysulfides, the mechanisms of the biosynthesis of H<sub>2</sub>S and polysulfides, the properties of the biosynthetic enzymes, and the regulation of enzymatic activity. The knockout mouse technique is a useful tool to determine new physiological functions, especially those of H<sub>2</sub>S and polysulfides. In the future, we shall take a closer look at symptoms in the human congenital deficiency of each enzyme. Further studies on the regulation of enzymatic activity by in vivo substances may be the key to finding new functions of H<sub>2</sub>S and polysulfides.

**Keywords:** cystathionine β-synthase; cystathionine γ-lyase; thiosulfate sulfurtransferase; H<sub>2</sub>S; 3-mercaptopyruvate sulfurtransferase; polysulfides

## 1. Enzyme Production of H<sub>2</sub>S and Polysulfides

Cystathionine β-synthase (CBS) was first reported to produce H<sub>2</sub>S and polysulfides by Abe and Kimura in 1996 [1]. Further, cystathionine γ-lyase (CGL) was reported by Hosoki et al. [2], and 3-mercaptopyruvate sulfurtransferase (MST) was reported by Shibuya et al. [3–5], Mikami et al. [6,7], Modin et al. [8], Yadav et al. [9], Kimura et al. [10], and Nagahara et al. [11]. Thiosulfate sulfurtransferase (TST) was reported by Mikami et al. [7] and Kimura et al. [10]. These enzymes catalyze a transsulfuration reaction from a sulfur-donor substrate to a sulfur acceptor substrate. Then, the persulfurated or polysulfurated substrate is reduced to produce H<sub>2</sub>S and polysulfides during this reaction.

On the other hand, Nagahara et al. [11] recently demonstrated in vitro that MST transfers a sulfur atom from 3-mercaptopyruvate (MP) to the catalytic site cysteine to form stable persulfide (polysulfide) as a reaction intermediate. It is interesting that as an alternative production process, thiol-containing compounds attack the persulfide (polysulfide) formed at the catalytic site and a new persulfide (polysulfide) molecule is formed at the thiol-containing compound. Then, dithiol is

reduced by thioredoxin (Trx) or dihydrolipoic acid to release H<sub>2</sub>S or polysulfides. This process may be autoreduction. Yadav et al. [10] performed enzyme kinetics analysis for human MST in the production process of hydrogen disulfide.

## 2. Physiological Functions of H<sub>2</sub>S and Polysulfides

Kimura reviewed the physiological activities of H<sub>2</sub>S and polysulfides in 2016 [12]. The functions of H<sub>2</sub>S and polysulfides are summarized in Tables 1 and 2, respectively, [1,2,13–32].

**Table 1.** Physiological function of H<sub>2</sub>S.

Function	Reference
Induction of long-term potentiation in the hippocampus as a synaptic model of memory	Abe and Kimura, 1996 [1]
Effect on smooth muscle relaxant activity	Hosoki et al., 1997 [2]
Protective action of nerve cells from oxidative stress	Kimura and Kimura, 2004 [13]
Regulation of insulin secretion	Yang et al., 2005 [14]; Kaneko et al., 2006 [15]
Oxygen sensor	Olson et al., 2006 [16]; Peng et al., 2010 [17]
Antiinfection	Zanardo et al., 2006 [18]
Protective action of myocardium and kidney from ischemia reperfusion injury	Elrod et al., 2007 [19]; Tripatara et al., 2008 [20]
Angiogenic effect	Cai et al., 2007 [21]; Papapetropoulos et al., 2009 [22]
Protection of retinal neurons from light-induced damage and apoptosis	Mikami et al., 2011b [7]
Regulation of endoplasmic reticulum stress	Krishnan et al., 2011 [23]
Bacterial resistance against antibiotics	Shatalin et al., 2011 [24]
Reduction of disulfide bonds in a ligand-binding domain of N-methyl-D-aspartic acid receptors	Kimura, 2013 [25]; 2015 [26]
Amplification of the activity of N-methyl-D-aspartic acid receptor upon activation by neurotransmitters	Kimura, 2015 [26]
Activation of H <sup>+</sup> -ATPase resulting in decrease of calcium influx into photoreceptor cells of the retina	Kimura, 2016 [12]

Sulfane-sulfur binding proteins (SSBPs), ubiquitous in cells and tissues, can be regarded as potentially H<sub>2</sub>S-releasing molecules when under proper redox conditions and affected by specific stimuli that induce their release. Three sulfurtransferases, i.e., CGL, MST, and TST, carry sulfane sulfur, which can be released as H<sub>2</sub>S/HS<sup>−</sup> [33]. The released H<sub>2</sub>S can be enzymatically oxidized to sulfane sulfur by sulfide quinone oxidoreductase with an acceptor of sulfane sulfur, such as GSH [34].

To understand the physiological function of sulfane sulfur, its levels in biological samples (tissues, cell cultures) were determined using the reaction with cyanide, and subsequently investigating the thiocyanate yield of the complex with Fe<sup>3+</sup>, which is detectable by spectrophotometry [35]. Although the method is not very sensitive, it showed that sulfane sulfur levels were quite similar in various animal tissues [36,37] and in murine macrophages despite stimulation with lipopolysaccharide and interferon- $\gamma$  [38]. Thus, an argument may be put forward about homeostasis of sulfane sulfur levels in biological systems. Moreover, a negative feedback regulation between CBS and CTH was suggested by Nandi and Mishra [39] and confirmed by Bronowicka et al. [38]. The adaptive cellular response to stimulation with both IFN $\gamma$  and LPS caused a decreased level of H<sub>2</sub>S-associated low CBS expression and increased CTH expression.

**Table 2.** Physiological function of polysulfides.

Function	Reference
Induction of calcium influx by activating a cation channel, subfamily A, and member 1 in astrocytes	Nagai et al., 2004 [27]; Kimura et al., 2013 [28]
Inhibition of tumor suppressor lipid phosphatase and tensin homolog by changing the protein to its oxidized form	Greiner et al., 2013 [29]
Upregulation of antioxidant genes, such as heme oxygenase 1 and glutamate cysteine ligase	Koike et al., 2013 [30]
Induction of long-term potentiation in the hippocampus due to activation of N-methyl-D-aspartic acid receptors	Kimura, 2015 [26]
Upregulation of antioxidant genes such as heme oxygenase 1 and glutamate cysteine ligase	Kimura, 2015 [26]
Decrease in toxic carbonyl stress in neuroblastoma cells	Koike et al., 2015 [31]
Induction of neural outgrowth and cell differentiation of neuroblastoma cells	Koike et al., 2016 [32]

The adaptive cellular response to electrophiles (electron-deficient species) represented by heavy metal ions [40] involves sulfane sulfur atoms of numerous SSBPs. Protection against electrophilic stresses involves persulfides rather than thiol groups because of their higher nucleophilicity [41]. Electrophiles are captured by reactive persulfide/polysulfide species, resulting in formation of their sulfur adducts [42]. In bovine aortic endothelial cells, CSE knockdown potentiated Cd-induced cytotoxicity but CSE overexpression provided protection [43]. In vivo experiments showed that CSE-knockout mice were sensitive to Cd-induced hepatotoxicity [44]. Adaptive changes in the activity and expression of CGL, MST, and TST in various frog tissues in response to exposure to lead, mercury, and cadmium confirmed the protective function of these enzymatic proteins against electrophilic stress [45,46].

### 3. Possible Production of Other Sulfur-Containing Substances, Sulfur Oxides

Physiological roles of sulfur dioxide were reported by Liu et al. [47] in 2016 and include vasorelaxation [48–50] as well as myocardial injury [48]. MALDI-TOF-MS analysis provided supporting evidence for sulfur oxide production (SO, SO<sub>2</sub>, and SO<sub>3</sub>) in the redox cycle of sulfane sulfur as a reaction intermediate of MST by Nagahara et al. [51] in 2012. The persulfurated catalytic site cysteine was oxidized to form Cys-thiosulfenate (Cys-Sγ-SO), Cys-thiosulfinate (Cys-Sγ-SO<sub>2</sub>), and Cys-thiosulfonate (Cys-Sγ-SO<sub>3</sub>). Reducing agents such as DTT and Trx produce sulfur oxides [51].

### 4. Knockout of H<sub>2</sub>S and Polysulfides-Producing Enzymes

Knockout (KO) technique is a good tool to clarify the physiological functions of proteins. Congenital deficiency of CBS causes hyperhomocysteinemia or homocystinuria in humans. Watanabe et al. produced CBS-KO mice [52], and the mice were afflicted with chronic renal dysfunction. The mice showed growth retardation and died within 5 weeks.

Congenital deficiency of CGL causes cystathioninuria in humans. CGL-KO mice were produced by Yang et al. [53], and the mice displayed low levels of H<sub>2</sub>S associated with hypertension.

Congenital deficiency of MST causes mercaptolactate-cysteine disulfiduria in humans. MST-KO mice were produced by Nagahara et al. [54]; however, mercaptolactate-cysteine disulfiduria has not been examined. Peleli et al. [55] recently reported that MST-KO mice were protected against ischemic reperfusion of the heart. Nasi et al. [56] reported that mice showed accelerated joint calcification

and osteoarthritis due to an increase in chondrocyte mineralization. Interestingly, these two findings indicate that MST demonstrates both good and bad effects on living organisms.

TST-KO mice have not been obtained; however, a double KO mouse for both TST and MST has been produced (unpublished data).

## 5. Regulation of Enzymatic Activity by In Vivo Substances

These four enzymes have been reported to regulate enzymatic activities in vivo. CBS activity is inhibited by CO [57], NO [58], L-cystathione [59], and L-homocysteine [60]. On the other hand, CBS activation by in vivo substances has not been reported. CGL activity is inhibited by acetoacetate [61], alanine [62], cysteine [63], glycine [62], serine [62],  $\text{Cd}^{2+}$  [64],  $\text{Cu}^{2+}$  [64],  $\text{H}_2\text{O}_2$  [61], and  $\text{O}_2$  [65]. It is interesting that oxidative stress inhibits CBS activity. However, CGL is activated by L-cysteine [66] and 2-mercaptoethanol (probably other reducing agents also reactivate) [62]. Thus, reducing conditions reactivate CBS. TST activity is inhibited by  $\text{Ca}^{2+}$  [67],  $\text{Zn}^{2+}$  [67],  $\text{Cu}^{2+}$  [68], oxaloacetate [69], pyruvate [69],  $\text{H}_2\text{S}$  [70],  $\text{SO}_3^{2-}$  [67],  $\text{SO}_4^{2-}$  [69], sulfide [71], sulfite [72], and  $\text{H}_2\text{O}_2$  [73]. Oxidative stress also inhibits TST activity. On the other hand, TST is activated by L-cysteine [74], glutathione [75], and reduced glutathione [75,76]. Thus, reducing conditions also reactivate TST. MST activity is inhibited by alpha-ketobutyrate [77], alpha-ketoglutarate [77], pyruvate [78], cysteine [78], sulfite [6], glutathione [78], and  $\text{H}_2\text{O}_2$  [79]. Oxidative stress also inhibits MST activity, while MST is activated by thioredoxin [79,80]. Thus, reducing conditions also reactivate MST. In the three enzymes, these enzymatic activities are regulated by the redox state.

## 6. Conclusions

Four cysteine-containing enzymes (CBS, CGL, MST, and TST) produce  $\text{H}_2\text{S}$  and polysulfides via the reduction of persulfurated or polysulfurated substrates. MST is also produced via the reduction of stable persulfide (polysulfide) formed at a catalytic-site cysteine as a reaction intermediate. Sulfur oxide can also be produced from a catalytic site cysteine of MST. These products play an important role in living organisms. Furthermore, studies using KO mice of each enzyme have clarified the physiological function of these enzymes, which cannot be assessed in wild-type animals. Regulation of enzymatic activity by in vivo substances may be related to the functions of  $\text{H}_2\text{S}$  and polysulfides.

**Author Contributions:** These authors contributed equally to this work. All authors have read and agreed to the published version of the manuscript.

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## References

1. Abe, K.; Kimura, H. The possible role of hydrogen sulfide as an endogenous neuromodulator. *J. Neurosci.* **1996**, *16*, 1066–1071. [[CrossRef](#)] [[PubMed](#)]
2. Hosoki, R.; Matsuki, N.; Kimura, H. The possible role of hydrogen sulfide as an endogenous smooth muscle relaxant in synergy with nitric oxide. *Biochem. Biophys. Res. Commun.* **1997**, *237*, 527–531. [[CrossRef](#)] [[PubMed](#)]
3. Shibuya, N.; Tanaka, M.; Yoshida, M.; Ogasawara, Y.; Togawa, T.; Ishii, K.; Kimura, H. 3-Mercaptopyruvate sulfurtransferase produces hydrogen sulfide and bound sulfane sulfur in the brain. *Antioxid. Redox Signal.* **2009**, *11*, 703–714. [[CrossRef](#)] [[PubMed](#)]
4. Shibuya, N.; Mikami, Y.; Kimura, Y.; Nagahara, N.; Kimura, H. Vascular endothelium expresses 3-mercaptopyruvate sulfurtransferase and produces hydrogensulfide. *J. Biochem.* **2009**, *146*, 623–626. [[CrossRef](#)]
5. Shibuya, N.; Koike, S.; Tanaka, M.; Ishigami-Yuasa, M.; Kimura, Y.; Ogasawara, Y.; Fukui, K.; Nagahara, N.; Kimura, H. A novel pathway for the production of hydrogen sulfide from D-cysteine in mammalian cells. *Nat. Commun.* **2013**, *4*, 1366. [[CrossRef](#)]

6. Mikami, Y.; Shibuya, N.; Kimura, Y.; Nagahara, N.; Ogasawara, Y.; Kimura, H. Thioredoxin and dihydrolipoic acid are required for 3-mercaptopyruvate sulfurtransferase to produce hydrogen sulfide. *Biochem. J.* **2011**, *439*, 479–485. [[CrossRef](#)]
7. Mikami, Y.; Shibuya, N.; Kimura, Y.; Nagahara, N.; Yamada, M.; Kimura, H. Hydrogen sulfide protects the retina from light-induced degeneration by the modulation of Ca<sup>2+</sup> influx. *J. Biol. Chem.* **2011**, *286*, 39379–39386. [[CrossRef](#)]
8. Modis, K.; Asimakopoulou, A.; Coletta, C.; Papapetropoulos, A.; Szabo, C. Oxidative stress suppresses the cellular bioenergetic effect of the 3-mercaptopyruvate sulfurtransferase/hydrogen sulfide pathway. *Biochem. Biophys. Res. Commun.* **2013**, *433*, 401–407. [[CrossRef](#)]
9. Yadav, P.K.; Yamada, K.; Chiku, T.; Koutmos, M.; Banerjee, R. Structure and kinetic analysis of H<sub>2</sub>S production by human mercaptopyruvate sulfurtransferase. *J. Biol. Chem.* **2013**, *288*, 20002–20013. [[CrossRef](#)]
10. Kimura, Y.; Toyofuku, Y.; Koike, S.; Shibuya, N.; Nagahara, N.; Lefer, D.; Ogasawara, Y.; Kimura, H. Identification of H<sub>2</sub>S<sub>3</sub> and H<sub>2</sub>S produced by 3-mercaptopyruvate sulfurtransferase in the brain. *Sci. Rep.* **2015**, *5*, 14774. [[CrossRef](#)]
11. Nagahara, N.; Koike, S.; Nirasawa, T.; Kimura, H.; Ogasawara, Y. Alternative pathway of H<sub>2</sub>S and polysulfides production from sulfurated catalytic-cysteine of reaction intermediates of 3-mercaptopyruvate sulfurtransferase. *Biochem. Biophys. Res. Commun.* **2018**, *496*, 648–653. [[CrossRef](#)] [[PubMed](#)]
12. Kimura, H. Physiological roles of hydrogen sulfide and polysulfides. *Folia Pharm. Jpn.* **2016**, *147*, 23–29. [[CrossRef](#)] [[PubMed](#)]
13. Kimura, Y.; Kimura, H. Hydrogen sulfide protects neurons from oxidative stress. *FASEB J.* **2004**, *8*, 1165–1167. [[CrossRef](#)] [[PubMed](#)]
14. Yang, W.; Yang, G.; Jia, X.; Wu, L.; Wang, R. Activation of KATP channels by H<sub>2</sub>S in rat insulin-secreting cells and the underlying mechanisms. *J. Physiol.* **2005**, *569*, 519–531. [[CrossRef](#)] [[PubMed](#)]
15. Kaneko, Y.; Kimura, Y.; Kimura, H.; Niki, I. L-Cysteine inhibits insulin release from the pancreatic  $\beta$ -cell. *Diabetes* **2006**, *55*, 1391–1397. [[CrossRef](#)]
16. Olson, K.R.; Dombkowski, R.A.; Russell, M.J.; Doellman, M.M.; Head, S.K.; Whitfield, N.L.; Madden, J.A. Hydrogen sulfide as an oxygen sensor/transducer in vertebrate hypoxic vasoconstriction and hypoxic vasodilation. *J. Exp. Biol.* **2006**, *209*, 4011–4023. [[CrossRef](#)]
17. Peng, Y.J.; Nanduri, J.; Raghuraman, G.; Souvannakitti, D.; Gadalla, M.M.; Kumar, G.K.; Snyder, S.H.; Prabhakar, N.R. H<sub>2</sub>S mediates O<sub>2</sub> sensing in the carotid body. *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 10719–10724. [[CrossRef](#)]
18. Zano, R.C.O.; Brancalione, V.; Distrutti, E.; Fiorucci, S.; Cirino, G.; Wallace, J.L. Hydrogen sulfide is an endogenous modulator of leukocyte-mediated inflammation. *FASEB J.* **2006**, *20*, 2118–2120. [[CrossRef](#)]
19. Elrod, J.W.; Calvert, J.W.; Morrison, J.; Doeller, J.E.; Kraus, D.W.; Tao, L.; Jiao, X.; Scalia, R.; Kiss, L.; Szabo, C.; et al. Hydrogen sulfide attenuates myocardial ischemia-reperfusion injury by preservation of mitochondrial function. *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 15560–15565. [[CrossRef](#)]
20. Tripathi, P.; Patel, N.; Collino, M.; Gallicchio, M.; Kieswich, J.; Castiglia, S.; Benetti, E.; Stewart, K.N.; Brown, P.A.; Yaqoob, M.M.; et al. Generation of endogenous hydrogen sulfide by cystathionine  $\gamma$ -lyase limits renal ischemia/reperfusion injury and dysfunction. *Lab. Invest.* **2008**, *88*, 1038–1048. [[CrossRef](#)]
21. Cai, W.J.; Wang, M.J.; Moore, P.K.; Jin, H.M.; Yao, T.; Zhu, Y.C. The novel proangiogenic effect of hydrogen sulfide is dependent on Akt phosphorylation. *Cardiovasc Res.* **2007**, *76*, 29–40. [[CrossRef](#)]
22. Papapetropoulos, A.; Pyriochou, A.; Altaany, Z.; Yang, G.; Marazioti, A.; Zhou, Z.; Jeschke, M.G.; Branski, L.K.; Herndon, D.N.; Wang, R.; et al. Hydrogen sulfide is an endogenous stimulator of angiogenesis. *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 21972–21977. [[CrossRef](#)]
23. Krishnan, N.; Fu, C.; Pappin, D.J.; Tonks, N.K. H<sub>2</sub>S-induced sulphydration of the phosphatase PTP1B and its role in the endoplasmic reticulum stress response. *Sci. Signal.* **2011**, *4*, ra86. [[CrossRef](#)]
24. Shatalin, K.; Shatalina, E.; Mironov, A.; Nudler, E. H<sub>2</sub>S: A universal defense against antibiotics in bacteria. *Science* **2011**, *334*, 986–990. [[CrossRef](#)]
25. Kimura, H. Physiological role of hydrogen sulfide and polysulfide in the central nervous system. *Neurochem. Int.* **2013**, *63*, 492–497. [[CrossRef](#)]
26. Kimura, H. Hydrogen sulfide and polysulfides as signaling molecules. *Proc. Jpn. Acad. Ser. B* **2015**, *91*, 131–159. [[CrossRef](#)]



27. Nagai, Y.; Tsugane, M.; Oka, J.I.; Kimura, H. Hydrogen sulfide induces calcium waves in astrocytes. *FASEB J.* **2004**, *18*, 557–559. [[CrossRef](#)] [[PubMed](#)]
28. Kimura, Y.; Mikami, Y.; Osumi, K.; Tsugane, M.; Oka, J.; Kimura, H. Polysulfides are possible H<sub>2</sub>S-derived signaling molecules in rat brain. *FASEB J.* **2013**, *27*, 2451–2457. [[CrossRef](#)] [[PubMed](#)]
29. Greiner, R.; Pálincás, Z.; Bäsell, K.; Becher, D.; Antelmann, H.; Nagy, P.; Dick, T.P. Polysulfides link H<sub>2</sub>S to protein thiol oxidation. *Antioxid Redox Signal.* **2013**, *19*, 1749–1765. [[CrossRef](#)] [[PubMed](#)]
30. Koike, S.; Ogasawara, Y.; Shibuya, N.; Kimura, H.; Ishii, K. Polysulfide exerts a protective effect against cytotoxicity caused by t-buthylhydroperoxide through Nrf2 signaling in neuroblastoma cells. *FEBS Lett.* **2013**, *587*, 3548–3555. [[CrossRef](#)]
31. Koike, S.; Shibuya, N.; Kimura, H.; Ishii, K.; Ogasawara, Y. Polysulfide promotes neuroblastoma cell differentiation by accelerating calcium influx. *Biochem. Biophys. Res. Commun.* **2015**, *459*, 488–492. [[CrossRef](#)] [[PubMed](#)]
32. Koike, S.; Kayama, T.; Yamamoto, S.; Komine, D.; Tanaka, R.; Nishimoto, S.; Suzuki, T.; Kishida, A.; Ogasawara, Y. Polysulfides protect SH-SY5Y cells from methylglyoxal-induced toxicity by suppressing protein carbonylation: A possible physiological scavenger for carbonyl stress in the brain. *NeuroToxicol.* **2016**, *55*, 13–19. [[CrossRef](#)] [[PubMed](#)]
33. Toohey, J.I. Sulphane sulphur in biological systems: A possible regulatory role. *Biochem. J.* **1989**, *264*, 625–632. [[CrossRef](#)] [[PubMed](#)]
34. Libiad, M.; Yadav, P.K.; Vitvitsky, V.; Martinov, M.; Banerjee, R. Organization of the human mitochondrial hydrogen sulfide oxidation pathway. *J. Biol. Chem.* **2014**, *289*, 30901–30910. [[CrossRef](#)] [[PubMed](#)]
35. Wood, J.L. Sulfane sulfur. *Methods Enzymol.* **1987**, *143*, 25–29. [[PubMed](#)]
36. Wróbel, M.; P Sura, Z.; Srebro, Z. Sulfurtransferases and the content of cysteine, glutathione and sulfane sulfur in tissues of the frog *Rana temporaria*. *Comp. Biochem. Physiol. Part B* **2000**, *125*, 211–217. [[CrossRef](#)]
37. Wróbel, M.; Czubak, J.; Bronowicka-Adamska, P.; Jurkowska, H.; Adamek, D.; Papla, B. Is development of high-grade gliomas sulfur-dependent. *Molecules* **2014**, *19*, 21350–21362. [[CrossRef](#)]
38. Bronowicka-Adamska, P.; Jurkowska, H.; Gawda, A.; Skalska, P.; Nazimek, K.; Marcinkiewicz, J.; Wróbel, M. Expression and activity of hydrogen sulfide generating enzymes in murine macrophages stimulated with lipopolysaccharide and interferon- $\gamma$ . *Mol. Biol. Rep.* **2019**, *46*, 2791–2798. [[CrossRef](#)]
39. Nandi, S.S.; Mishra, P.K. H<sub>2</sub>S and homocysteine control a novel feedback regulation of cystathionine beta synthase and cystathionine gamma lyase in cardiomyocytes. *Sci. Rep.* **2017**, *7*, 3639. [[CrossRef](#)]
40. Nishida, M.; Kumagai, Y.; Ihara, H.; Fujii, S.; Motohashi, H.; Akaike, T. Redox signaling regulated by electrophiles and reactive sulfur species. *J. Clin. Biochem. Nutr.* **2016**, *58*, 91–98. [[CrossRef](#)]
41. Yasuhiro, S.; Yoshito, K. Sulfane Sulfur in Toxicology: A Novel Defense System Against Electrophilic Stress. *Toxicol. Sci.* **2019**, *170*, 3–9.
42. Nishida, M.; Sawa, T.; Kitajima, N.; Ono, K.; Inoue, H.; Ihara, H.; Motohashi, H.; Yamamoto, M.; Suematsu, M.; Kurose, H.; et al. Hydrogen sulfide anion regulates redox signaling via electrophile sulphydration. *Nat. Chem. Biol.* **2012**, *8*, 714–724. [[CrossRef](#)] [[PubMed](#)]
43. Shinkai, Y.; Masuda, A.; Akiyama, M.; Xian, M.; Kumagai, Y. Cadmium-mediated activation of the HSP90/HSF1 pathway regulated by reactive persulfides/polysulfides. *Toxicol. Sci.* **2017**, *156*, 412–421. [[CrossRef](#)] [[PubMed](#)]
44. Akiyama, M.; Shinkai, Y.; Unoki, T.; Shim, I.; Ishii, I.; Kumagai, Y. The capture of cadmium by reactive polysulfides attenuates cadmium-induced adaptive responses and hepatotoxicity. *Chem. Res. Toxicol.* **2017**, *30*, 2209–2217. [[CrossRef](#)]
45. Kaczor, M.; Sura, P.; Bronowicka-Adamska, P.; Wróbel, M. Exposure to lead in water and cysteine non-oxidative metabolism in *Pelophylax ridibundus* tissues. *Aquat. Toxicol.* **2013**, *127*, 72–77. [[CrossRef](#)]
46. Kaczor, M.; Sura, P.; Wróbel, M. Changes in activity of three sulfurtransferases in response to exposure to cadmium, lead and mercury ions. *J. Environ. Prot.* **2013**, *4*, 19–28. [[CrossRef](#)]
47. Liu, J.; Huang, Y.; Chen, S.; Tang, C.; Jin, H.; Du, J. Role of endogenous sulfur dioxide in regulating vascular structural remodeling in hypertension. *Oxid. Med. Cell Longev.* **2016**, *2016*, 4529060. [[CrossRef](#)]
48. Du, S.X.; Jin, H.F.; Bu, D.F.; Zhao, X.; Geng, B.; Tang, C.S.; Du, J.B. Endogenously generated sulfur dioxide and its vasorelaxant effect in rats. *Acta. Pharm. Sin.* **2008**, *29*, 923–930. [[CrossRef](#)]
49. Meng, Z.; Zhang, H. The vasodilator effect and its mechanism of sulfur dioxide-derivatives on isolated aortic rings of rats. *Inhal. Toxicol.* **2007**, *19*, 979–986. [[CrossRef](#)]

50. Wang, Y.K.; Ren, A.J.; Yang, X.Q.; Wang, L.G.; Rong, W.F.; Tang, C.S.; Yuan, W.J.; Lin, L. Sulfur dioxide relaxes rat aorta by endothelium-dependent and -independent mechanisms. *Physiol. Res.* **2009**, *58*, 521–527.
51. Nagahara, N.; Nirasawa, T.; Yoshii, T.; Niimura, Y. Is novel signal transducer sulfur oxide involved in the redox cycle of persulfide at the catalytic site cysteine in a stable reaction intermediate of mercaptopyruvate sulfurtransferase? *Antioxid Redox Signal.* **2012**, *16*, 747–753. [[CrossRef](#)] [[PubMed](#)]
52. Watanabe, M.; Osada, J.; Aratani, Y.; Kluckman, K.; Reddick, R.; Malinow, M.R.; Maeda, N. Mice deficient in cystathionine  $\gamma$ -synthase: Animal models for mild and severe homocyst(e)inemia. *Proc. Natl. Acad. Sci. USA* **1995**, *92*, 1585–1589. [[CrossRef](#)] [[PubMed](#)]
53. Yang, G.; Wu, L.; Jiang, B.; Yang, W.; Qi, J.; Cao, K.; Meng, Q.; Mustafa, A.K.; Mu, W.; Zhang, S.; et al. H<sub>2</sub>S as a physiologic vasorelaxant: Hypertension in mice with deletion of cystathionine gamma-lyase. *Science* **2008**, *322*, 587–590. [[CrossRef](#)] [[PubMed](#)]
54. Nagahara, N.; Nagano, M.; Ito, T.; Shimamura, K.; Akimoto, T.; Suzuki, H. Antioxidant enzyme, 3-mercaptopyruvate sulfurtransferase-knockout mice exhibit increased anxiety-like behaviors: A model for human mercaptolactatecysteine disulfiduria. *Sci. Rep.* **2013**, *3*, 1986. [[CrossRef](#)]
55. Peleli, M.; Bibli, S.I.; Li, Z.; Chatzianastasiou, A.; Varela, A.; Katsouda, A.; Zukunft, S.; Bucci, M.; Vellecco, V.; Davos, C.H.; et al. Cardiovascular phenotype of mice lacking 3-mercaptopyruvate sulfurtransferase. *Biochem. Pharm.* **2020**. in print. [[CrossRef](#)]
56. Nasi, S.; Ehriochou, D.; Chatzianastasiou, A.; Nagahara, N.; Papapetropoulos, A.; Bertrand, J.; Cirino, G.; So, A.; Busso, B. The protective role of the 3-mercaptopyruvate sulfurtransferase (3-MST)-hydrogen sulfide (H<sub>2</sub>S) pathway against experimental osteoarthritis. *Arthritis Res.* **2020**. in print. [[CrossRef](#)]
57. Taoka, S.; West, M.; Banerjee, R. Characterization of the heme and pyridoxal phosphate cofactors of human cystathionine beta-synthase reveals nonequivalent active sites. *Biochemistry* **1999**, *38*, 2738–2744. [[CrossRef](#)]
58. Taoka, S.; Banerjee, R. Characterization of NO binding to human cystathionine beta-synthase: Possible implications of the effects of CO and NO binding to the human enzyme. *J. Inorg. Biochem.* **2001**, *87*, 245–251. [[CrossRef](#)]
59. Aitken, S.M.; Kirsch, J.F. Kinetics of the yeast cystathionine beta-synthase forward and reverse reactions: Continuous assays and the equilibrium constant for the reaction. *Biochemistry* **2003**, *42*, 571–578. [[CrossRef](#)]
60. Belew, M.S.; Quazi, F.I.; Willmore, W.G.; Aitken, S.M. Kinetic characterization of recombinant human cystathionine beta-synthase purified from *E. coli*. *Protein Expr. Purif.* **2009**, *64*, 139–145. [[CrossRef](#)]
61. Manna, P.; Gungor, N.; McVie, R.; Jain, S.K. Decreased cystathionine- $\gamma$ -lyase (CSE) activity in livers of type 1 diabetic rats and peripheral blood mononuclear cells (PBMC) of type 1 diabetic patients. *J. Biol. Chem.* **2014**, *289*, 11767–11778. [[CrossRef](#)]
62. Chu, L.; Ebersole, J.L.; Kurzban, G.P.; Holt, S.C. Cystalsin, a 46-kDa L-cysteine desulfhydrase from *Treponema denticola*: Biochemical and biophysical characterization. *Clin. Infect. Dis.* **1999**, *28*, 442–450. [[CrossRef](#)] [[PubMed](#)]
63. Braunstein, A.E.; Goryachenkova, E.V. The beta-replacement-specific pyridoxal-P-dependent lyases. *Adv. Enzym. Relat. Areas Mol. Biol.* **1984**, *56*, 1–89.
64. Matsuo, Y.; Greenberg, D.M. A crystalline enzyme that cleaves homoserine and cystathionine. III. Coenzyme resolution, activators, and inhibitors. *J. Biol. Chem.* **1959**, *234*, 507–515. [[PubMed](#)]
65. Olson, K.R.; Healy, M.J.; Qin, Z.; Skovgaard, N.; Vulesevic, B.; Duff, D.W.; Whitfield, N.L.; Yang, G.; Wang, R.; Perry, S.F. Hydrogen sulfide as an oxygen sensor in trout gill chemoreceptors. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **2008**, *29*, R669–R680. [[CrossRef](#)]
66. Wróbel, M.; Lewandowska, I.; Bronowicka-Adamska, P.; Paszewski, A. The level of sulfane sulfur in the fungus *Aspergillus nidulans* wild type and mutant strains. *Amino Acids* **2009**, *37*, 565–571. [[CrossRef](#)] [[PubMed](#)]
67. Westley, J. Rhodanese. *Adv. Enzym. Relat. Areas Mol. Biol.* **1973**, *39*, 327–368.
68. Lee, C.Y.; Hwang, J.H.; Lee, Y.S.; Cho, K.S. Purification and characterization of mouse liver rhodanese. *J. Biochem. Mol. Biol.* **1995**, *28*, 170–176.
69. Oi, S. Inhibition of rat liver rhodanese by di-, tricarboxylic, and alpha-keto acids. *J. Biochem.* **1975**, *78*, 825–834. [[CrossRef](#)]
70. Picton, R.; Eggo, M.C.; Merrill, G.A.; Langman, M.J.; Singh, S. Mucosal protection against sulphide: Importance of the enzyme rhodanese. *Gut* **2002**, *50*, 201–205. [[CrossRef](#)]

71. Ramasamy, S.; Singh, S.; Taniere, P.; Langman, M.J.; Eggo, M.C. Sulfide-detoxifying enzymes in the human colon are decreased in cancer and upregulated in differentiation. *Am. J. Physiol. Gastrointest Liver Physiol.* **2006**, *291*, G288–G296. [[CrossRef](#)] [[PubMed](#)]
72. Vazquez, E.; Gazzaniga, S.; Polo, C.; Batlle, A. Mitochondrial and cytosolic rhodanese from liver of DAB-treated mice. III. Inhibition kinetic studies. *Cancer Biochem. Biophys.* **1997**, *15*, 285–293. [[PubMed](#)]
73. Nandi, D.L.; Horowitz, P.M.; Westley, J. Rhodanese as a thioredoxin oxidase. *Int J. Biochem. Cell Biol.* **2000**, *32*, 465–473. [[CrossRef](#)]
74. Anosike, E.O.; Jack, A.S. A comparison of some biochemical properties of liver thiosulphate sulphurtransferase from guinea pig (*Lepus caniculus*) & albino rat. *Indian J. Biochem. Biophys.* **1982**, *19*, 13–16.
75. Turkowsky, A.; Blotevogel, K.H.; Fischer, U. Properties of a soluble thiosulfate sulfur transferase (rhodanese) of the marine methanogen *Methanosarcina frisia*. *FEMS Microbiol. Lett.* **1991**, *81*, 251–256. [[CrossRef](#)]
76. Vandenbergh, P.A.; Berk, R.S. Purification and characterization of rhodanese from *Acinetobacter calcoaceticus*. *Can. J. Microbiol.* **1980**, *26*, 281–286. [[CrossRef](#)]
77. Porter, D.W.; Baskin, S.I. The effect of three alpha-keto acids on 3-mercaptopyruvate sulfurtransferase activity. *J. Biochem. Toxicol.* **1996**, *11*, 45–50. [[CrossRef](#)]
78. Vachek, H.; Wood, J.L. Purification and properties of mercaptopyruvate sulfur transferase of *Escherichia coli*. *Biochim. Biophys. Acta* **1972**, *258*, 133–146. [[CrossRef](#)]
79. Nagahara, N.; Katayama, A. Post-translational regulation of mercaptopyruvate sulfurtransferase via a low redox potential cysteine-sulfenate in the maintenance of redox homeostasis. *J. Biol. Chem.* **2005**, *280*, 34569–34576. [[CrossRef](#)]
80. Nagahara, N.; Yoshii, T.; Abe, Y.; Matsumura, T. Thioredoxin-dependent enzymatic activation of mercaptopyruvate sulfurtransferase. An intersubunit disulfide bond serves as a redox switch for activation. *J. Biol. Chem.* **2007**, *282*, 1561–1569. [[CrossRef](#)]



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